Exhibit M

EXPERIMENTAL THERAPEUTICS

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Synergy of Navelbine-Taxol Combination Treatment in Two Human Breast Cancer Cell Lines. David J. Adams, Division of Cell Biology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

The interaction of Navelbine® (vinorelbine) with Taxol® (paclitaxel) was evaluated by the method of Chou and Talalay in two human breast cancer cell lines, MCF-7 and MDA-MB-231, which are models for the early and progressive forms of disease, respectively. Tumor cells were exposed concurrently or sequentially to drugs for three or four days, followed by 24 h recovery in drug-free medium. Viable (by cellular metabolism) or total cell number (by protein or DNA content) was then determined employing standard curves from drug-treated cells. The results demonstrate: (1) Taxol and Navelbine are synergistic when administered concurrently in a molar ratio of 0.1 to 10 (Taxol:Navelbine); (2) synergy occurs over a range of activity (20-80% inhibition of cell growth); (3) active concentrations of the drugs in combination are clinically achievable (e.g., IC_{50}'s = 1-10 nM); and (4) the combination is antagonistic when cells are exposed to Taxol for 24 h prior to the addition of Navelbine. Overall, these findings provide a rationale for the clinical evaluation of concurrent combination chemotherapy with Navelbine and Taxol in human breast cancer.

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Preclinical activity of Navelbine® and Taxol drug combinations. Knick, V.C., Eberwein, D.J. & Miller, C.G. Division of Cell Biology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Navelbine® (NVB), 5'-nor-anhydrovinblastine, and Taxol (TAX) (NSC 125973) have demonstrated clinical activity against ovarian, breast and non-small cell lung carcinoma. NVB acts to depolymerize microtubules and TAX acts to stabilize polymerized tubulin into microtubule bundles. Since NVB and TAX attack the microtubular synthetic process of the mitotic spindle at two distinct sites, we investigated the interaction of the two drugs in a binary combination. Dose-ranging and scheduling studies were conducted against intraperitoneally (IP) implanted P388 leukemia. Both agents were given IP and optimal activity was noted on a q4dx3 schedule with NVB administered thirty to sixty minutes before TAX. In two experiments, NVB alone at 10 mg/kg produced weight loss (10 and 15%), early drug-related deaths (4/5 and 2/5) and increased life span (ILS) (18 and 145%) while TAX alone at 38 mg/kg was non-toxic with 55% and 84% ILS. There were no 60 day survivors with either agent alone. The NVB-TAX combination significantly reduced the weight loss or provided a slight weight gain, eliminated early drug-related deaths and improved the ILS to 118 and 227 with 4/5 and 1/5 60 day tumor free survivors respectively. Studies to confirm these results against human xenograft tumors are ongoing.

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Docetaxel (RP 56976, Taxotere®) efficacy as a single agent or in combination against mammary tumors in mice. Bissery, M.C., Vrignaud, P., Bayssas, M., and Lavelle, F. Rhône-Poulenc Rorer S.A., 94403 Vitry-sur-Seine, France.

Docetaxel (T) is a new antitumor agent undergoing Phase II clinical trials with promising activity in breast cancer (Proc. ASCO 12: 27, 1993). It was evaluated against 4 mouse mammary tumors MA13/C, MA16/C, MA17/A, MA44. Early stage MA13/C and MA16/C were found highly sensitive to T with 0% T/C (median tumor weight of the treated over the control x 100) and complete regressions of advanced stage disease were obtained. MA44 was modestly sensitive to T (T/C=39%) and MA17/A was not sensitive (T/C=59%). T was further evaluated in combination with doxorubicin (A), 5-fluorouracil (F), cyclophosphamide (C), mitomycin C (MC), vincristine (VCR), vinblastine (VLB) and vinorelbine (NVB) against MA13/C using simultaneous administration. The maximum tolerated dose of each drug that could be administered in combination without additional toxicity was 60-70% for T-A, T-F, T-C, T-MC and 80-100% for T-VCR, T-VLB and T-NVB. These data are of importance for the design of future combination trials in human breast cancer.

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Antiproliferative effects of the retinoid fenretinide (4HPR) on human ovarian carcinoma cell lines. Supino, R., Clerici, M., Formelli, F. Istituto Nazionale Tumori, 20133 Milan, Italy

Recently we showed that 4HPR, a synthetic retinoid currently tested clinically, inhibits the "in vivo" growth of the human ovarian carcinoma IGROV-1 and enhances the antitumor activity of cisplatin (DDP) against this tumor. The effects of 4HPR on ovarian tumors have been further studied in four cell lines "in vitro": A2780, IGROV-1, SW626 and OVCA432. The inhibition of cell growth was concentration dependent and reversible after drug removal. A2780 was the most sensitive line. Its growth was inhibited by 50% by .9 μM 4HPR, a concentration which is pharmacologically achievable in patients. The other cell lines were 10 times less sensitive. Following 4HPR treatment, A2780 showed an increase of cell number in $S\text{-}G_2$ phase, of p53 expression and of apoptotic events. The cell cycle was not affected in the other cell lines. The effects of the combination of 4HPR with DDP were tested on A2780 and IGROV-1. A2780 was also more sensitive to DDP (ID_{50}: .2 μM in A2780, .6 μM in IGROV-1). The addition of an ID_{30} of 4HPR to varying concentrations of DDP resulted in a greater than additive effect in both lines. These results indicate that the antitumor activity of 4HPR and the enhancement of the cytotoxic activity of DDP are correlated to direct growth inhibitory effects on ovarian carcinoma cell lines.

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Combination 5-Fluorouracil (FU), folinic acid (FA) and alpha-interferon 2B (IFN) in advanced gastric cancer.
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FU has cytotoxic activity in gastric cancer cell lines. Intracellular thymidilate synthase (TS) levels and cytotoxicity of FU are inversely correlated. Modulation of FU with FA or IFN has shown increased and prolonged cytotoxic effects of FU. With single agent FU therapy clinical responses of up to 20 % (CR+PR) were observed in gastric cancer. A phase II study was initiated to evaluate toxicity and effects of [illegible] modulation of FU with FA and IFN in patients (pts) with locally advanced/metastatic gastric cancer. Schedule: FA 500 mg/qm 2 hour infusion 1x/week, IFN 6 M.U. s.c. 3x/week, FU 500 mg/qm bolus i.v. 1x/week. Of 63 consecutive pts with progressive disease, all (21 females, 42 males) were evaluable for response and toxicity. Median age was 55,6 years (21-80), median Karnofsky performance status was 80 % (70-100). Sites of measurable disease were inoperable primary tumor/local recurrence (18), liver metastasis (16), lymph nodes (24) and peritoneum (16). 1pt had paraneoplastic hyperfibrinolytic coagulopathy. Results: Clinical CR were achieved in 1/63 pts, PR in 19/63 pts, MR in 10/63 pts, tumor stabilization in 17/63 pts and 2/63 pts had progressive disease. Median duration of response was 6 months (mo), median progression-free intervals 4.5 mo, median survival time was 10 mo for all pts, 12 mo for CR/PR pts. 6/8 CR pts has lymph node metastasis only, 2/8 had additional liver metastasis. Paraneoplastic hyperfibrinolysis was controlled for 5 mo under treatment without further symptomatic therapy. Toxicity: 1/63 pts had WHO grade 4 toxicity (diarrhea), 3/63 pts had WHO grade 3 toxicity (nausea 1, diarrhea 2). Except for 1 treatment limiting grade 4 toxicity, no modifications of dose or schedule due to toxicity were required. 36/44 pts experienced significant reduction of tumor related pain under treatment. Conclusion: Biochemical modulation of FU with FA and IFN demonstrates superior effects compared to single agent FU in advanced gastric cancer. Moderate toxicity, outpatient treatment setting and high response rates of tumor related pain contribute to an effective palliation. Further accrual including assessment of TS expression in cancer cells under treatment is ongoing to evaluate the significance of this marker for treatment decisions in gastric cancer.

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Combined effects of taxol and a vitamin D agonist against breast and ovarian cancer cells. Saunders, D.E., Christensen, C., Wappler, N.L., Lawrence, W.D., Malone, J.M., Malviya, V.K., and Deppe, G. Depts of Pathology and OB/GYN, Wayne State University, Detroit, MI 48201

Taxol has demonstrated effectiveness against breast and ovarian malignancies, and its effectiveness may be increased by combining it with other anticancer agents. We have previously shown that NIH:OVCAR3 human ovarian and MCF-7 human breast cancer cells were effectively growth inhibited when taxol was combined with calcitriol, the most active natural vitamin D metabolite. The current study involved *in vitro* evaluation of the effectiveness of combining taxol with EB1089, a potent second-generation, analog of vitamin D. OVCAR3 cells were exposed 3 days to taxol (0-4.5 ng/ml) alone and in combination with EB1089 (0-100 nM), followed by measurement of growth inhibition with an MTT dye reduction assay. EB1089 substantially enhanced taxol's effects and isobolographic analysis showed that the interaction between the 2 agents ranged from additivity to synergism. MCF-7 cells were exposed to 0-9 ng/ml taxol and 0-9 nM EB1089. Addition of 0-9 nM EB1089 to 0-9 ng/ml taxol enhanced taxol's effectiveness against MCF-7 cells. Taxol and EB1089 interacted additively at 1:1 and 3:1 ratios. These experiments suggest that the addition of EB1089 may increase the effectiveness of taxol against OVCAR3 ovarian and MCF-7 breast cancer cells.